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13. ABSTRACT ***(Maximum 200 Words)***

Tumor angiogenesis is the common pathophysiological factor of both primary tumors and distant metastases. Therefore, the ablation of the angioigenesis may have a key role to stop tumor progression. In this regard we and others have shown that, when given systemically, normal endothelial progenitors (EPs) circulate and localize into areas of active angiogenesis. In this proposal, we will evaluate the employment of these EPs as cellular vehicles for gene delivery into primary and metastatic carcinoma of the prostate. To this end, we will apply EPs genetically modified ex vivo with a toxin gene for cell-mediated molecular chemotherapy. Thus, if localization occurs in the areas of tumor angiogenesis as proposed, expression of the toxin gene inside the EPs could induce death in these toxin-expressing cells and their neighbors, thus abrogating angiogenesis, and therefore potentially inducing regression of the tumor thereafter by vascular deprivation.

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INTRODUCTION:

Cellular vehicles represent an extremely novel vector approach to accomplish cancer gene therapy overcoming existing drawbacks of viral and non-viral vectors. In this regard Mesenchymal Progenitor Cells, (MPC) possess key capacities suggesting their candidacy as cellular vehicles. Thus, the realization of specific aims of current proposal will broaden a field of existing gene delivery approaches, addressing a key issue relevant to prostate cancer gene therapy. We hypothesize that MPC represent an attractive candidate as cell vector for gene delivery based on intrinsic properties of this cell population: they are readily harvestable, expandable and can function as syngeneic delivery vehicles. We further hypothesize that favorable intrinsic properties of MPC could be supplemented with vector properties, such as efficient loading of cells with therapeutic genes and homing (native or engineered) to the tumor sites. Thus, we seek to explore the potential of MPC to function as cellular vehicles for gene-based therapy of cancer of the prostate.

BODY:

To establish the feasibility of a cell-based strategy, we investigated major vector characteristics of several cell populations:

a) The ability to efficiently express the gene of choice - to be genetically loaded
b) The ability to efficiently deliver therapeutic payloads to the target tissue/organ after systemic or local administration due to either native or engineered tropism – to be targeted.

Primary cells can be efficiently transduced *ex vivo* by using adenoviral vectors.
Adenoviral vectors (Ad) are the most effective means for *ex vivo* gene delivery, providing that the cell population has a sufficient level of the adenovirus receptors - CAR (Coxsackie Adenovirus Receptors). Relatively low transduction efficiency for primary cell cultures such as lymphocytes, dendritic cells, hematopoetic stem cells as well as primary tumor cells has been reported. MPC cultures also have been proved as difficult for transduction with Ad vectors. For instance it has been documented that high MOIs (250-1000 pfu/cell) are required to achieve a transduction efficiency of 20-25%. Indeed our preliminary data **(Fig 1)** and the others show that MPC has a negligible level of CAR expression, which may explain the inefficiency of transduction. One way to overcome the low CAR phenotype limitation of MPC is to utilize a CAR-independent entry of the Ad vectors. For this purpose a variety of Ad vectors with modified tropism (AdRGD, Ad5/3) have been created and extensively tested at the Gene Therapy Center at UAB for *ex vivo* transduction of primary cells of different origin. In our experiments for optimization of MPC transduction, we found that Ad with enhanced tropism for cellular integrins (AdRGD) can indeed substantially augment efficiency of MPC transduction **(Fig 2)**.

In essence these data show that Ad vectors with expanded tropism can substantially enhance the efficiency of MPC transduction, thereby providing the means to introduce an essential property of cellular vehicle – the ability to be genetically loaded. **Therefore MPC can achieve sufficient level of expression of either reporter or therapeutic genes, allowing to study their biodistribution after injection, or biologic effect in an animal model of prostate cancer.**

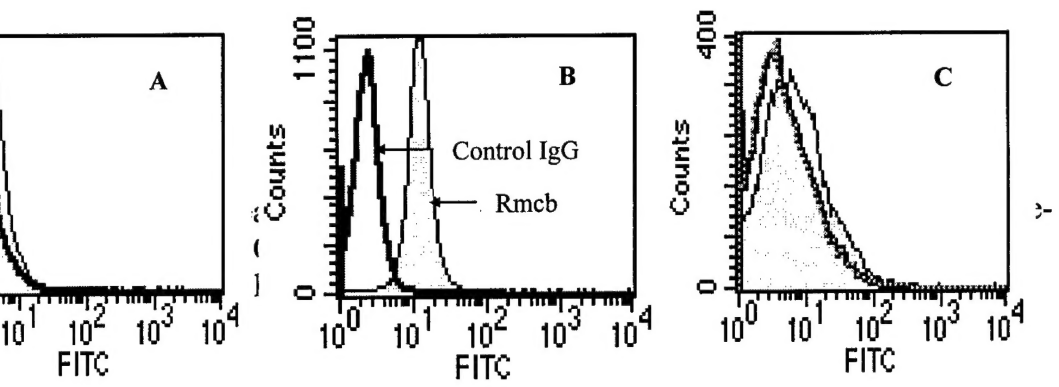

Fig1. FITC (HEL

Fig1. CAR expression on MPC measured by flow cytometry with anti-CAR (Rmcb)/ anti -mouse-FITC Ab.(A)-CAR-negative cells (CHO) was used as negative control, (B)- CAR-positive cells (HELA), (C)-MPC. MPC has very low level of CAR expression.

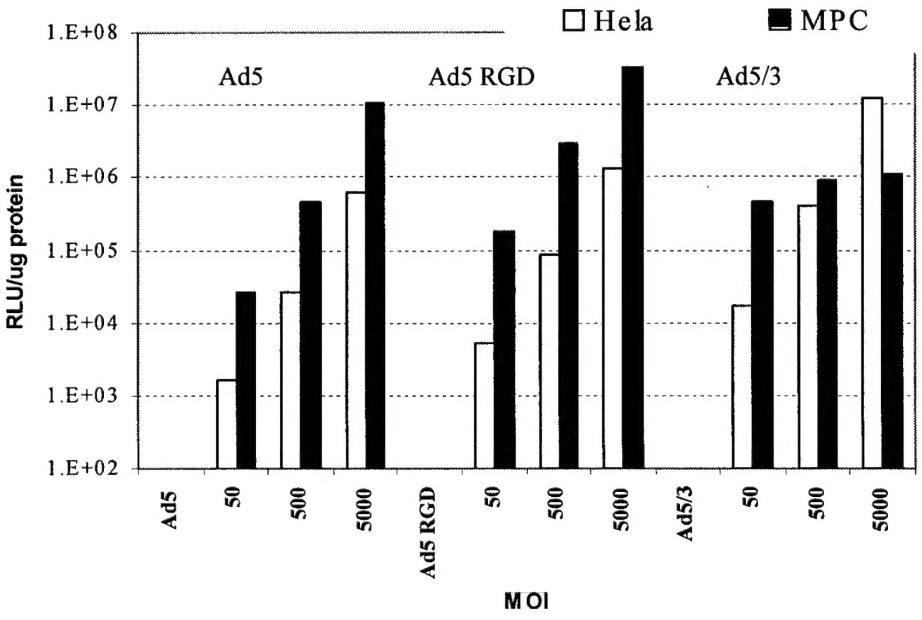

Fig 2. Increase in transduction efficiency of MPC with modified adenoviral vectors. Cells were transduced with adenoviral vectors Ad5, Ad5RGD, Ad5/3 carrying a luciferase reporter gene at 50, 500, 5000 vp/cell. After 24 hr luciferase expression was measured in relative light units and normalized to the amount of protein as determined by BioRad Assay (RLU/□g protein).

Additional binding properties can be introduced to the cellular vehicles by using single chain Ab (sFv) as a targeting moiety.

The native homing property of cultured MPC after reimplantation into the body by different routes still remains a subject of investigation. Assuming that targeting strategies widely employed for viral and non-viral vectors may have utility in the context of cellular vectors, we seek to introduce a targeting moiety to MPC to artificially enhance homing of these cells to the tumor.

Single chain antibodies (scFv) represent an attractive class of ligands, which can be employed as a targeting moiety. ScFv-mediated targeting has been shown in a variety of applications mostly in context of immunologic retargeting of viral and non-viral vectors. The feasibility of using scFv as a binding moiety in a cellular context has been shown in our group by designing an artificial receptor for fiber-modified adenoviruses. Two artificial receptors have been tested, extracellular domains of which are derived from a single-chain antibody with binding specificities either to Ad5 fiber protein (knob domain) or to a 6-His Tag **(Fig 3).**

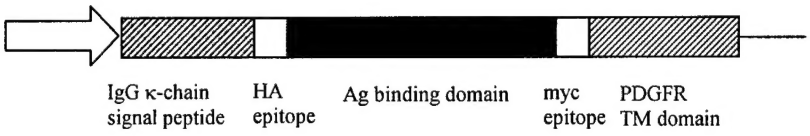

Fig 3. Schematic diagram of the cassette used for expression of the surface-displayed artificial receptors. The Ig k-chain leader directs the receptor to the cell surface and the PDGFR transmembrane domain anchors the receptor in the plasma membrane. The HA epitope permits detection by immunohistochemistry. Ag-binding domain can be easily switched to obtain different binding specificity.

Expression of an anti-knob scFv on the surface of cells non-permissive for Ad infection restores ability of those cells to be infected by adenovirus **(Fig 4).**

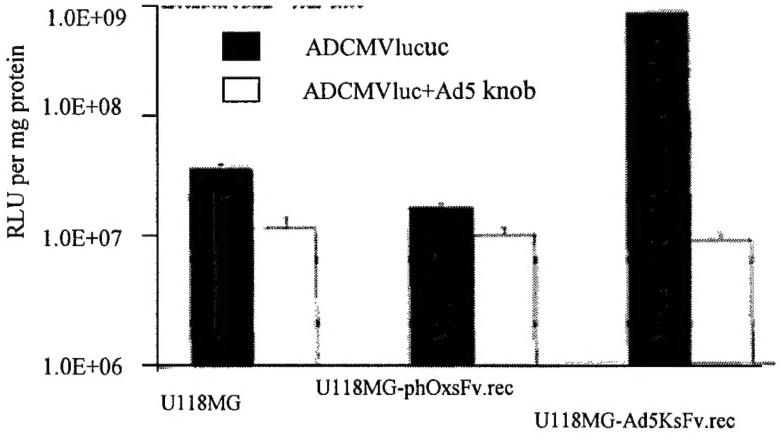

Fig 4. Ad5KsFv.rec functions as an artificial receptor for Ad5 infection of non-permissive U118MG cells. U118MG human glioma cells were transfected with pAd5KsFv.rec or the control plasmid pHook and individual single-cell clones were isolated. (A) Parental U118MG cells, or stably transfected U118MG-phOxsFv.rec and U118MG-Ad5KsFv.rec cells were preincubated with PBS or Ad5 knob prior to infection with AdCMVLuc. Results are the mean of triplicate experiments.

It has also been demonstrated that Ad having 6-His genetically incorporated in its fiber protein was able to infect cells through interactions between fiber-6His and anti-6His scFv on the cell surface. **Thus, these data suggest that scFvs presented on the cellular surface retain binding specificity to its ligand, allowing the use of this property for target of applications vis à vis prostate cancer gene therapy.**

KEY RESEARCH ACCOMPLISHMENTS:

- Demonstration that mesenchymal progenitor cells (MPCs) could function as a stem cell substitute for endothelial progenitors (EPs) in context of the original cell vehicle concept.
- Demonstration that MPC could be transduced with adenoviral vectors to thereby contain an anti-cancer agent.
- Demonstration that MPC could be transduced to express T-body moieties to facilitate their ability to target cancer cells *in vivo*.
- Demonstration of anti-tumor efficacy of pro-apotosis molecules as candidate anti-cancer gene for MPC loading.
- Establishment of MPC's as candidate cell vehicles for prostate cancer.
- Establishment of MP's as target cells which can be loaded with genetic anti-cancer agents.

REPORTABLE OUTCOMES: N/A

CONCLUSION:

We have shown that several primary cell cultures of MPC can be efficiently transduced *ex vivo* by using adenoviral vectors. We have demonstrated that low transduction efficiency of primary cell cultures with Ad vectors due to adenoviral receptor deficiency can be successfully overcomed by exploiting adenoviral vectors with modified CAR-independent entry mechanism. **Thus, cells intended to be exploited as vehicles can be efficiently loaded *ex vivo* with reporter or therapeutic genes.**

Further, we have shown that targeting moieties can be expressed in MPC. This property will potentially enhance their utility for target cell specific delivery in the context of prostate cancer gene therapy approaches.